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Rediscovery of *Crocus biflorus* var. *estriatus* (Iridaceae) and its taxonomic characterisation

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Abstract

The Italian endemic *Crocus biflorus* usually shows white or lilac flowers with three-to-five striking violet longitudinal stripes on the outer tepals, but unstriped plants were recorded in the past. These plants were originally described as *C. annulatus* subvar. *estriatus*, and subsequently recombined as a variety of *C. biflorus*. The rediscovery of such plants in Toscana gave us the opportunity to clarify their systematic relationships, so that we typified the name, and performed karyological and ITS analyses. These plants share the same chromosome number ($2n = 2x = 8$) and ITS sequence with *C. biflorus* s. str.

Keywords

Chromosome number, Herbert, Italian endemics, ITS, typification

Introduction

The genus *Crocus* L. (Iridaceae) consists of about 200 recognized species, ranging from western Europe and north-western Africa to western China, with a centre of diversity in the Balkan Peninsula and in Turkey (Mathew 1982, Harpke et al. 2015, 2016, Peruzzi 2016).

This genus is currently subdivided in two sections (*C.* sect. *Crocus* and *C.* sect. *Nudiscapus* B.Mathew) and 15 series (Harpke et al. 2016). Among them, *C.* ser. *Biflori* B.Mathew is characterised by corms with membranous or coriaceous tunics generally

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splitting into rings, trilobe style, and winter flowering (Mathew 1982). The type species of *C.* ser. *Biflori* is *Crocus biflorus* Mill., an Italian endemic (Harpke et al. 2016) occurring in all regions, with the exception of Valle d'Aosta and Sardegna (Bartolucci et al. 2018).

Although *C. biflorus* usually shows white or lilac flowers with three-to-five striking violet longitudinal stripes on the outer tepals (Maw 1886, Mathew 1982, Harpke et al. 2016), unstriped plants were recorded in the past (Mathew 1982). These plants were described by W. Herbert (1778–1847) as *C. annulatus* Herb. subvar. *estriatus* Herb. (Herbert 1841), and subsequently recombined by P.A. Tchichatscheff (1812–1890) as a variety of *C. biflorus* (Tchichatscheff 1860).

Herbert (1841) quoted this plant "*prope Florentia*" (near Firenze) for Toscana (Italy), and later Maw (1881), added the Chartreuse of Firenze and the Botanical Garden of Roma (Lazio) as further localities. Then, the name *C. biflorus* var. *estriatus* was used by Fiori (1908), who did not specify any locality and stated that this variety can be found in the same area of striped plants. Other authors of Italian floras (Parlatore 1860, Arcangeli 1882, Fiori 1923, Pignatti 1982, Colasante 2017) did not mention any name for plants showing unstriped tepals. On the contrary, Arcangeli (1882) used the name *C. biflorus* var. *lineatus* (Jan.) Nyman to indicate regularly striped plants.

During a field survey in Empoli (Firenze), we discovered a population of *C. biflorus* showing all the individuals marked by flowers with unstriped tepals (Fig. 1). This discovery gave us the opportunity to clarify the systematic relationships of these plants, so that we typified the name *Crocus annulatus* subvar. *estriatus* and we performed karyological and ITS analyses.

Material and methods

Typification of the name

According to Stafleau & Cowan (1979), the Herbert Herbarium is preserved at K, whereas other specimens cited in the protologue (i.e. Herbarium of J.D. Hooker and Herbarium of A.B. Lambert) are preserved at BM, E, G, K, and MANCH (herbarium acronyms follow Thiers 2018). Hence, for typification purposes, we searched for original material in these herbaria and carried out bibliographic investigations starting from the information published by Herbert (1841) along with the protologue.

Karyological and molecular analyses

Plants were collected in Empoli (Firenze, Toscana) (Fig. 1). The population, uniformly made by plants with unstriped flowers, consisted of about 210 individuals and covered an area of approximately 110 m^2.

Figure 1. *Crocus biflorus* var. *estriatus* from the epitype locality in Empoli, Firenze (photos by L. Peruzzi).

For the karyological study, young developing ovaries were pre-treated with 0.4% colchicine for 3 hours and then fixed in Carnoy fixative solution for 1 hour. Then, after hydrolysis in HCl 1N at 60° C, the material was stained in leuco-basic fuchsine. Meristematic cells were squashed on a slide, adding a drop of acetic orceine, and then analysed under light microscope for the detection of metaphasic plates.

Genomic DNA was extracted from about 10 mg of silica-dried leaf material with the DNeasy Plant DNA Extraction Kit (Qiagen) according to the protocol of the manufacturer. DNA concentration and quality were afterwards checked on 0.8% agarose gels. The ITS region (ITS1, 5.8S rDNA, ITS2) was amplified using the primers ITS-A and ITS-B (Blattner 1999), according to Harpke et al. (2013). Both strands of the PCR products were directly sequenced with Applied Biosystems BigDye Terminator technology on an ABI 3730xl automatic DNA sequencer using the primers from PCR amplifications. Forward and reverse sequences from each directly sequenced amplicon were inspected, manually edited where necessary, and combined in a single consensus sequence. The sequence obtained in this study was deposited in the EMBL nucleotide database under accession number LS991340. The derived ITS sequences were compared to the already published sequence data of *C. biflorus* s. str. (Harpke et al. 2016).

Geographical distribution

In order to investigate the distribution of *Crocus biflorus* var. *estriatus*, we studied specimens from the two main Tuscan herbaria of FI and PI, including the separate collections PI-ARC, PI-CAR, PI-GUAD, PI-PASS, and PI-PELL. Finally, using QGIS 2.18 software, we georeferenced all the specimens and literature data in order to draw a distribution map.

Specimina visa

ITALY. **Tuscany**. Dans les prairies des Cascines, Florence, 26 February 1850, *T. Caruel* (PI-CAR); Prairie des Cascine pres Florence, Italie, 20 February 1861, *T. Caruel* (PI); Cascine, Florence, February 1870, *A. Archbald* (FI); Firenze a Boboli, March 1872, *Scafferi* (FI); abunde in pratis ambulacri florentini "le Cascine", 20 February 1874, *E. Levier* (FI); in prati seccis ambulacrum le Cascine prope Florentiam, 13 February 1876, *J. Arcangeli* (PI-ARC); Cascine di Firenze nel prato misto con la [?], February 1880, *U. Martelli* (FI); Environs de Florence (Italie): prairies surtout dans les cascines, March 1888, *H. Groves* (FI); Firenze nei prati delle Cascine, 19 March 1898, *U. Martelli* (PI); Cascine prope Florentiam, in pratis, 4 March 1903, *G. Stefanini* (FI); prope Florentiam (Firenze), vulgatus in pratis loco dicto Le Cascine, 10 March 1904, *R. Pampanini et P. Baccarini* (FI, PI-GUAD); prope Florentiam alle Cascine, prato al Quercione (ora al Calcio), 9 January 1916, *G. Gervasi* (FI); Empoli (Firenze), sul Lungarno poco prima di giungere alla Tinaia provenendo da Empoli (WGS84: 43.734473 N, 10.964509 E), incolto con prevalenza di *Dactylis glomerata* e *Artemisia verlotiorum*, 27 m s.l.m., 28 January 2018, *L. Peruzzi et F. Roma-Marzio* (PI n° 011333–011336); Empoli (Firenze), presso la foce dell'Orme (WGS84: 43.726944 N, 10.951719 E), incolto con prevalenza di *Dactylis glomerata* e *Artemisia verlotiorum*, 22 m s.l.m., 28 January 2018, *L. Peruzzi et F. Roma-Marzio* (PI n° 011337). **Campania**. Valle di San Rocco, 24 April 1834, *A. Orsini* (FI); *ibidem*, February 1869, *coll. illeg.* (PI-CAR); Valle di S. Rocco, 1893, *Avellino* (FI).

Typification of the name

***Crocus biflorus* Mill. var. *estriatus* (Herb.) Tchich., Asie Min., Bot. 2: 520 (1860)**

≡*Crocus annulatus* Herb. subvar. *estriatus* Herb., Botanical Magazine 67: t. 3862. (1841)
≡*Crocus annulatus* Herb. var. *estriatus* (Herb.) Herb., J. Hort. Soc. London 2: 286 (1847)
≡*Crocus biflorus* Mill. var. *estriatus* (Herb.) Maw *comb. superfl.* Gard. Chron. 16: 749 (1881)

Lectotype (here designated): *Crocus pusillus*. Edward's Bot. Reg., 23: plate 1987 [icon] (image available at: https://biodiversitylibrary.org/page/240669). **Epitype** (here designated): Empoli (Firenze), sul Lungarno poco prima di giungere alla Tinaia provenendo da Empoli (WGS84: 43.734473 N, 10.964509 E), incolto con prevalenza di *Dactylis glomerata* e *Artemisia verlotiorum*, 27 m s.l.m., 28 January 2018, *L. Peruzzi et F. Roma-Marzio* (PI n° 011333; isoepitypes PI n° 011334; PI n° 011335; PI n° 011336. Image of epitype and isoepitypes are available at: http://herbarium.univie.ac.at/database/search.php?collection=PI).

In the protologue, Herbert (1841) refers to an iconography of "*Crocus pusillus*" published by Lindley (1837) and quotes two specimens: a first one collected in "*Tiflim*" (probably Tiflis, an old name for Tiblisi the capital of Georgia) in the Her-

barium of J.D. Hooker (1817–1911), and a second one from an unspecified eastern locality ("*Spec. orientale*") collected by P.S. Pallas (1741–1811) and preserved in the Herbarium of A.B. Lambert (1761–1842).

In all the investigated herbaria we did not find specimens recognisable as original material (Art. 9.3 of ICN, Turland et al. 2018), so that we selected as lectotype the illustration published by Lindley (1837), i.e. the only available original material. This illustration clearly shows cultivated plants with unstriped tepals, whose original provenance is indicated as "native of the southern parts of Italy" by Lindley (1837). Anyway, to avoid any possible ambiguity concerning the application of this name (Art. 9.8 of ICN), we also designated an epitype collected in Empoli, not far for Firenze, a locality explicitly cited in the protologue by Herbert (1841).

Karyology, molecular systematics, and distribution

Our karyological analysis revealed a diploid chromosome count $2n = 2x = 8$ (Fig. 2), whereas ITS sequence is 665 bp long and is identical to that of regularly striped *Crocus biflorus* individuals from its type locality.

Concerning the distribution of unstriped plants, according to herbarium and literature data they are recorded for three different localities in the province of Firenze (Toscana), for a single locality in Campania near Napoli, and for the Botanical Garden of Roma, albeit the only currently confirmed locality is that from Empoli, Firenze (Fig. 3).

Figure 2. *Crocus biflorus* var. *estriatus*: metaphasic plate with $2n = 2x = 8$ chromosomes. Scale bar: 10 μm.

Discussion

Unstriped plants share the same chromosome number ($2n = 2x = 8$) with *C. biflorus* s. str. (Brighton et al. 1973, Baldini 1993, Illuminati et al. 1995, Campo et al. 1999, Peruzzi and Cesca 2002). Brandizzi and Grilli Caiola (1997) published a $2n = 18$ chromosome count, putatively obtained from plants collected near Roma; however, as pointed by Bedini et al. (2010 onwards) this count is certainly erroneous, very likely due to a typo in the original paper, where indeed the basic chromosome number is reported as $x = 4$. In addition, ITS sequence is identical to that of *C. biflorus* s. str.

Figure 3. Distribution map of *Crocus biflorus* var. *estriatus*, based on historical herbarium specimens (blue dots) and historical literature (green dots). The blue star represents the newly discovered population (Empoli), which is also the only currently confirmed.

individuals from type locality, again from Toscana (Harpke et al. 2016). These results confirm that unstriped plants fall within the variability of *C. biflorus*, so that a taxonomic treatment at varietal level is appropriate.

Given that *C. biflorus* is an Italian endemic species (Harpke et al. 2016, Bartolucci et al. 2018), after the typification of the name *C. annulatus* subvar. *estriatus* on Italian material, we can assume that the unstriped plants from eastern Mediterranean localities cited in the protologue by Herbert (1841) certainly refer to other taxa. In fact, Harpke et al. (2016) demonstrated that all the 23 previously recognized subspecies of *C. biflorus*, ranging from Balkan Peninsula to Caucasus and Iran, represent independent evolutionary lineages, which should be treated at species level.

Considering that many crocuses are known as popular ornamentals (Harpke et al. 2013), the hypothesis that the spread in Italy of *C. biflorus* var. *estriatus* may have been favoured/caused by human activity is consistent with the distribution of unstriped plants in Italy, since they have been found always not far from urban centres (Herbert 1841, Maw 1881).

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